Recent Advances on the Nutritional Effects Associated with the Use of Garlic as a Supplement

Cholesterol-Lowering Effect of Garlic Extracts and Organosulfur Compounds: Human and Animal Studies[1]

Yu-Yan Yeh[2] and Lijuan Liu

Department of Nutrition, The Pennsylvania State University, University Park, PA 16802

ABSTRACT The medicinal use of garlic dates back thousands of years, but there was little scientific support of its therapeutic and pharmacologic properties until recently. In the past decade, the cancer-protective effects of garlic have been well established by epidemiologic studies and animal experiments. However, the cardiovascular-protective properties of garlic are less well understood. In particular, despite the reported hypocholesterolemic effect of garlic, the mechanism of the effect is unclear. In a recent randomized, double-blind, placebo-controlled intervention study, we showed that aged garlic extract (AGE) supplementation was effective in lowering plasma concentration of total cholesterol by 7% and LDL cholesterol by 10% in hypercholesterolemic men compared with subjects consuming a placebo. Supplementation of AGE in animal diets similarly reduced plasma concentrations of total cholesterol and triacylglycerol by 15 and 30%, respectively. In subsequent experiments using cultured rat hepatocytes, we found 44–87% inhibition of cholesterol synthesis by the water-extractable fraction (WEF), methanol-extractable fraction (MEF) and petroleum ether–extractable fraction (PEF) of fresh garlic, and Kyolic (liquid form of AGE). These observations suggested that hydrophilic and hydrophobic compounds of garlic are inhibitory to cholesterol synthesis. Because *S*-allylcysteine (SAC) alone was less potent than Kyolic, which contains SAC and other sulfur compounds, a maximal inhibition appears to require a concerted action of multiple compounds of garlic. In a series of experiments, we further characterized the inhibitory potency of individual water-soluble and lipid-soluble compounds of garlic. Among water-soluble compounds, SAC, *S*-ethylcysteine (SEC), and *S*-propylcysteine (SPC) inhibited cholesterol synthesis by 40–60% compared with 20–35% by γ-glutamyl-*S*-allylcysteine (GSAC), γ-glutamyl-*S*-methylcysteine (GSMC) and γ-glutamyl-*S*-propylcysteine (GSPC). Lipid-soluble sulfur compounds (i.e., diallyl sulfide, diallyl disulfide, diallyl trisulfide, dipropyl sulfide and dipropyl trisulfide) at low concentrations (0.05–0.5 mol/L) slightly (10–15%) inhibited cholesterol synthesis but became highly cytotoxic at high concentrations (1.0–4.0 mol/L). All water-soluble compounds, except *S*-allylmercaptocysteine, were not cytotoxic, judging from the release of cellular lactate dehydrogenase into the culture medium. Taken together, the results of our studies indicate that the cholesterol-lowering effects of garlic extract, such as AGE, stem in part from inhibition of hepatic cholesterol synthesis by water-soluble sulfur compounds, especially SAC. J. Nutr. 131: 989S–993S, 2001.

KEY WORDS: • *garlic* • *organosulfur compounds* • *cholesterol* • *triacylglycerol* • *hepatocyte*

Medicinal use of garlic (*Allium sativum*) has existed for centuries (Dausch 1990, Han 1993), but there was little scientific support of its therapeutic and pharmacologic properties until recently. Epidemiologic studies in the past 10 years have revealed an inverse relationship between garlic consumption and the incidence of certain forms of cancer, including stomach, colon and laryngeal cancers (Buiatti et al. 1991, Mei et al. 1989, Steinmetz et al. 1994, Sumiyoshi and Wargovich 1990, Zheng et al. 1992). Animal studies have further substantiated the cancer-protective properties of garlic and various garlic preparations. Studies have shown that garlic extract and its constituents were effective in reducing the incidence of chemically induced mouse colon tumors (Sumiyoshi and Wargovich 1990) and rat mammary tumors (Amagase and Milner 1993, Lin et al. 1994, Liu et al. 1992, Schaffer et al. 1996). Organosulfur compounds of garlic also inhibited the growth of the human tumor cell lines HCT-15 (colon), SK MEL-2 (skin) and A549 (lung) in vitro (Sakamoto et al. 1997, Sundaram and Milner 1996). In addition, garlic has been shown to possess antithrombotic (Srivastava and Tyagi 1993), antiplatelet aggregation and antioxidative properties (Kiesewetter et al. 1993, Sendl et al. 1992, Yamasaki et al. 1994), and to stimulate the phagocytotic function of macrophage and lymphocyte proliferation (Tadi et al. 1990).

[1] Presented at the conference "Recent Advances on the Nutritional Benefits Accompanying the Use of Garlic as a Supplement" held November 15–17, 1998 in Newport Beach, CA. The conference was supported by educational grants from Pennsylvania State University, Wakunaga of America, Ltd. and the National Cancer Institute. The proceedings of this conference are published as a supplement to *The Journal of Nutrition*. Guest editors: John Milner, The Pennsylvania State University, University Park, PA and Richard Rivlin, Weill Medical College of Cornell University and Memorial Sloan-Kettering Cancer Center, New York, NY.

[2] To whom correspondence should be addressed. E-mail: yyy1@psu.edu.

The cardiovascular-protective effects of garlic have also been evaluated extensively in recent years. In animal experiments, garlic extracts have been shown to lower plasma lipid and cholesterol in rats (Chang and Johnson 1980, Chi 1982, Itokawa et al. 1973, Mathew et al. 1996, Yeh and Yeh 1994), rabbits (Bordia et al. 1975, Bordia and Verma 1980), chickens (Qureshi et al. 1983a, Qureshi et al. 1983b) and swine (Qureshi et al. 1987). Moreover, a number of intervention studies have similarly shown that garlic and garlic preparations significantly reduced plasma lipids, especially total cholesterol and LDL cholesterol in humans (Arora and Arora 1981, Bordia 1981, Jain et al. 1993, Lau et al. 1987, Steiner et al. 1996, Yeh et al. 1997, Zimmerman and Zimmerman 1990). Aside from the reported antiplatelet aggregation and antithrombotic action, garlic reduced blood pressure (Ernst 1987, Silagy and Neil 1994a, Steiner et al. 1996) and stimulated fibrinolytic activity (Arora et al. 1981, Ernst 1987). Two meta-analyses of randomized, placebo-controlled human studies confirmed the hypocholesterolemic effects of garlic (Silagy and Neil 1994b, Warshafsky et al. 1993). The analyses further detected that the extent of the cholesterol-lowering properties of garlic differed markedly from one study to another (Silagy and Neil 1994b, Warshafsky et al. 1993). It was estimated from the five randomized clinical trials that hypercholesterolemic patients treated with garlic had a mean plasma cholesterol concentration that was 9% lower than that of patients treated with placebo (Warshafsky et al. 1993). Silagy and Neil (1994b), on the other hand, concluded from the analysis of 17 human studies that plasma cholesterol concentrations of the subjects treated with garlic were 12% lower than those receiving placebo. Furthermore, the two analyses detected a wide range of decrease in mean plasma cholesterol concentrations (i.e., 6–53 mg/dL) among the studies. However, garlic supplementation has been shown not to decrease plasma cholesterol concentrations in human studies by Simons et al. (1995), Berthold et al. (1998) and Isaacsohn et al. (1998). Although the reasons for the inconsistent observations are not readily apparent, it is worthwhile to note that garlic contains a variety of organosulfur compounds, amino acids, vitamins and minerals (Block 1985). Some of the sulfur compounds such as allicin, ajoene, S-allylylcysteine (SAC),[3] diallyl disulfide (DADS), S-methylcysteine sulfoxide, and S-allylcysteine sulfoxide may be responsible for the therapeutic properties of garlic (Chi et al. 1982). Despite the fact that the mechanisims primarily responsible for the hypocholesterolemic action of garlic are uncertain at present, the composition and quantity of the sulfur components of different garlic preparations used in various studies could account in part for the inconsistent findings. Other contributing factors may include the subject recruitment, duration of experiment, dietary control, lifestyle and methods of lipid analyses (Silagy and Neil 1994b, Warshafsky et al. 1993).

The mechanism by which garlic or garlic preparations reduce plasma lipids has not been fully investigated. Animal studies, however, have shown that garlic supplementation in the diet depressed the hepatic activities of lipogenic and cholesterogenic enzymes such as malic enzyme, fatty acid synthase, glucose-6 phosphate dehydrogenase (Chi 1982, Chi et al. 1982, Qureshi et al. 1983a) and 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase (Qureshi et al. 1983a, 1983b and 1987). It is, therefore, reasonable that the hypocholesterolemic effect of garlic may stem in part from impaired cholesterol synthesis. In fact, we observed recently that garlic extracts that contained various sulfur compounds effectively decreased the plasma concentration of cholesterol, resulting possibly from an inhibition of hepatic cholesterol synthesis (Yeh and Yeh 1994).

The objectives of this article were to review the data on the cholesterol-lowering properties of garlic extracts in humans and animals and to summarize in vitro studies aimed at identifying the active sulfur compounds of garlic responsible for inhibition of cholesterol synthesis observed in our laboratories (Liu and Yeh 2000, Yeh and Yeh 1994, Yeh et al. 1997).

Human studies. In view of the potential confounding variables mentioned above, we designed a double-blind, randomized, placebo-controlled intervention study of free-living hypercholesterolemic subjects. Men (n = 34; 48.2 ± 0.8 y old) with plasma cholesterol concentration between 220 and 285 mg/dL were recruited into the study. The detailed account of the subjects and experimental design were described previously (Yeh et al. 1997). Briefly, the subjects were divided randomly into two groups (n = 17/group) to receive either garlic extract or placebo as dietary supplement for 5 mo. The subjects were asked to adhere strictly to their normal food habits and maintain healthy lifestyles. Each subject of the garlic group consumed daily 9 capsules, each containing 800 mg of aged garlic extract (AGE), whereas the placebo subjects took 9 capsules, each containing 800 mg of a common food ingredient. The capsules were prepared and provided by Wakunaga of America (Mission Viejo, CA). Plasma lipids were determined at the baseline period and 2, 4 and 5 mo after treatment. To ensure reliable quantitative measurements of plasma lipids, the regular quality controls of the Centers for Disease Control with known values of total cholesterol, HDL cholesterol and triacylglycerol were used as references in routine assays.

The mean baseline plasma total cholesterol concentrations were 246 ± 5 and 243 ± 5 mg/dL for the garlic and placebo groups, respectively (Yeh et al. 1997). Plasma concentrations of all lipids tested remained unchanged 2 mo after the supplementation of either AGE or placebo. Five months after the supplementation of AGE, the mean plasma concentration of total cholesterol was 7% (18 mg/dL) lower than the baseline value (**Fig. 1**A). Similarly, AGE supplementation reduced the mean plasma LDL cholesterol concentration by 10% (17 mg/dL) from its baseline level of 162 ± 4 mg/dL during the same time period (Fig. 1B). Although plasma concentrations of total and LDL cholesterol began to decrease by mo 4 of AGE supplementation, the changes were not significant. In contrast, mean plasma concentrations of total and LDL cholesterol were not altered in the group supplemented with the placebo for as long as 5 mo. At the conclusion of the study, i.e., 5 mo after the supplementation, mean plasma concentrations of total and LDL cholesterol of the AGE-treated group were 17 and 21 mg/dL lower, respectively, than those of the placebo-treated group. Plasma concentrations of HDL cholesterol and triacylglycerol remained constant throughout the study in the subjects regardless of the treatment.

Diet plays an important role in modulating plasma cholesterol. For example, excessive energy intakes increase hepatic production of very low density lipoprotein (VLDL), which serves as the precursor of LDL in the blood circulation (Grundy 1986), whereas dietary cholesterol and fat, especially

[3] Abbreviations used: AGE, aged garlic extract; DADS, diallyl disulfide; DAS, diallyl sulfide; DATS, diallyl trisulphide; DPDS, dipropyl disulfide; DPS, dipropyl sulfide; GSAC, γ-glutamyl-S-allylcysteine; GSMC, γ-glutamyl-S-methylcysteine; GSPC, γ-glutamyl-S-propylcysteine; HMG-CoA, 3-hydroxy-3-methyl-glutaryl-CoA; IC_{50}, 50% inhibitory concentration; LDH, lactate dehydrogenase; MAS, methyl allylsulfide; MEF, methanol-extractable fraction; PEF, petroleum ether-extractable fraction; SAAC, S-allyl acetylcysteine; SAC, S-allylcysteine; SAMC, S-allylmercaptocysteine; SASA, S-allyl sulfonylalanine; SEC, S-ethylcysteine; SMC, S-methylcysteine; SPC, S-propylcysteine; VLDL, very low-density lipoprotein; WEF, water-extractable fraction.

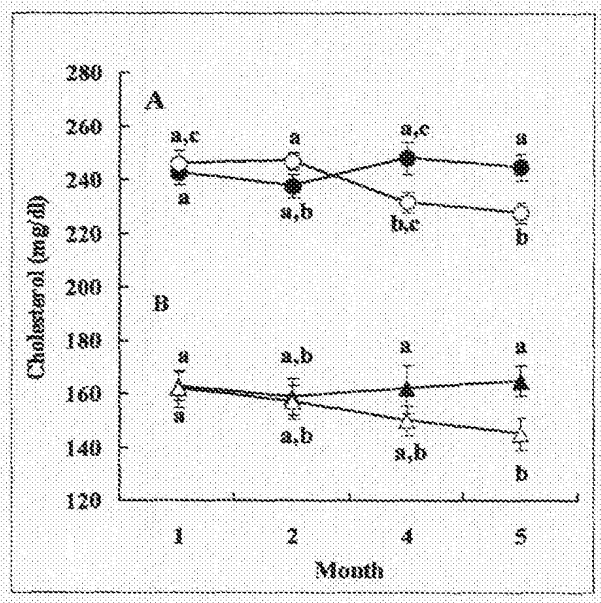

FIGURE 1 Reduction of plasma concentrations of total and LDL cholesterol by aged garlic extract supplementation in hypercholesterolemic men. *Panel A* shows plasma total cholesterol; *panel B* represents plasma LDL cholesterol. Closed circles and triangles represent placebo group; open circles and triangles represent the group supplemented with aged garlic extract. Values are means ± SEM, n = 16 or 17. Values without a common superscript are significantly different at $P < 0.05$.

saturated fat, cause plasma concentration of cholesterol to rise by down-regulating LDL receptor synthesis (Dietschy et al. 1993, Spady and Dietschy 1988). To assess whether the changes in plasma concentrations of cholesterol seen in the subjects receiving garlic supplementation could be associated with their diets, we analyzed 3-d food records taken during the course of the study. The analyses revealed that there were no differences in daily intakes of total energy, total fat, saturated and unsaturated fat, cholesterol, carbohydrate, protein and fiber at the baseline period, or at 2 and 4 mo after supplementation of either AGE or placebo (Yeh et al. 1997). There was no difference in nutrient intakes determined at the three time points between the two groups. Consistent with the constant energy intakes, there was no change in body weight or body mass index throughout the study, suggesting that the subjects seemed to adhere to their habitual lifestyles. These observations led us to conclude that the cholesterol-lowering effects demonstrated in this study could not be attributed to dietary modification but rather to the daily supplementation of the garlic extract.

Animal studies. The mechanism underlying the hypocholesterolemic action of AGE is not clear from our human study. Previous studies by other investigators have shown that the lipid-lowering effects of various garlic extracts were accompanied by depressed activities of lipogenic and cholesterogenic enzymes (Chi 1982, Chi et al.1982, Qureshi et al. 1983a, 1983b and 1987). Prompted by these findings, we conducted in vitro experiments using cultured rat hepatocytes to determine whether garlic decreases cholesterol synthesis (Yeh and Yeh 1994). Before the in vitro study, an animal feeding experiment was undertaken to confirm the lipid-lowering effect of garlic reported in different animal species (Chang and Johnson 1980, Chi 1982, Chi et al. 1982, Itokawa et al. 1973, Kamanna and Chandrasekhara 1982). Two groups of male Sprague-Dawley rats were fed either an AIN 76-diet containing 20 g/100 g fat, or a similar diet supplemented with 2 g/100 g AGE. Four weeks after the feeding, plasma concentrations of total cholesterol and triacylglycerol of the AGE-supplemented rats were 15 and 30% lower, respectively, than those of the nonsupplemented counterparts (**Fig. 2**). This finding led to subsequent experiments that used cultured rat hepatocytes to evaluate the possible role of garlic in cholesterol biosynthesis.

Cholesterol synthesis was determined by measuring the incorporation of [2-^{14}C]acetate into cholesterol (Yeh and Yeh 1994). A variety of garlic extracts were tested. These included the water-extractable fraction (WEF), methanol-extractable fraction (MEF) and petroleum ether-extractable fraction (PEF) of fresh garlic, Kyolic, a liquid form of AGE, and SAC. The rates of [2-^{14}C]acetate incorporation into cholesterol were depressed 44, 56 and 64% by MEF, PEF and WEF, respectively, suggesting that lipid-soluble as well as water-soluble components of garlic were effective in inhibiting cholesterol production (**Fig. 3**). These findings were in agreement with a previous study showing the inhibitory effect of water-soluble garlic extracts on cholesterol synthesis by cultured hepatocytes and HepG2 cells (Gebhardt 1993). These data further demonstrated that water-soluble compounds are more potent in inhibiting cholesterol synthesis than lipid-soluble compounds. Kyolic contains primarily water-soluble compounds, especially SAC, and a small amount of lipid-soluble sulfides (Weinberg et al. 1993). The incubation of hepatocytes with Kyolic at a concentration equivalent to 0.4 mmol/L SAC reduced the rate of cholesterol synthesis by 87%. On the other hand, when SAC at 2.0 mmol/L concentrations was incubated with the cells, the rate of cholesterol synthesis was decreased by only ~25% compared with the controls. A similar extent of the inhibition was achieved by a low concentration of Kyolic (i.e., 0.05 mmol/L SAC equivalent). The results strongly suggest that cholesterol synthesis is inhibited by a group of compounds that is either hydrophilic or hydrophobic in nature. The data further indicate that maximal inhibition of cholesterol synthesis requires a concerted action of multiple components of garlic.

In addition to the inhibition on cholesterol synthesis, WEF, MEF and PEF also depressed triacylglycerol synthesis as indi-

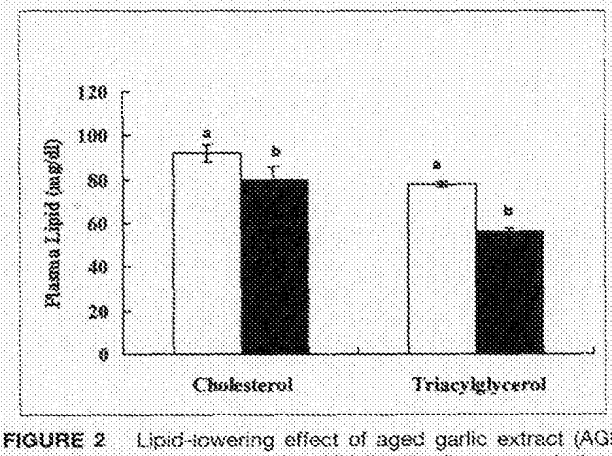

FIGURE 2 Lipid-lowering effect of aged garlic extract (AGE) in rats. Two groups of rats were fed AIN-76 diet (i.e., control diet, AIN 1977) or the same diet supplemented with 2 g/100 g AGE for 4 wk. At the end of the feeding, the fasting plasma cholesterol and triacylglycerol concentrations were determined. Open bars and closed bars represent the control and AGE-supplemented rats, respectively. Values are means ± SEM, n = 5. Values without a common superscript are significantly different at $P < 0.05$.

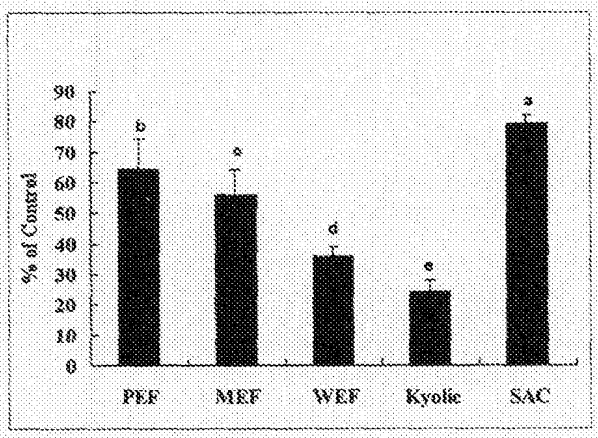

FIGURE 3 Inhibition of cholesterol synthesis by aged garlic extracts in cultured rat hepatocytes. Rat hepatocytes were maintained in Dulbecco's modified Eagle's medium supplemented with 5.6 mmol/L glucose, 10% fetal bovine serum and antibiotics (100 u penicillin/mL and 100 μg streptomycin/mL). Cells were incubated with the petroleum ether extractable fraction (PEF), methanol extractable fraction (MEF) and water extractable fraction (WEF) of fresh garlic at 1.25 g/L, Kyolic at 0.4 mmol/L and S-allylcysteine (SAC) at 2.0 mmol/L. The rates of [2-^{14}C]acetate incorporation into cholesterol ranged from 121 to 158 pmol acetate/(mg protein · 4 h) for the control group. Values are means ± SEM for three experiments. Values without a common superscript are significantly different at $P < 0.05$.

cated by a 9–14% reduction of [2-^{3}H]glycerol incorporation into triacylglycerol. The inhibition of triacylglycerol formation was apparent only when [2-^{3}H]glycerol was incubated in the presence of acetate but not fatty acid (e.g., oleic acid). This, together with the observed inhibition of [2-^{14}C]acetate incorporation into fatty acids by WEF, MEF, PEF and Kyolic, suggests that the garlic extracts impair triacylglycerol synthesis by inhibiting fatty acid production. Overall, the results of the study pointed out that the cholesterol-lowering action of garlic might be attributed in part to depressed cholesterol synthesis by the liver. The triacylglycerol-lowering effect of garlic, on the other hand, might be explained in part by its inhibitory action on fatty acid synthesis.

Active garlic components and cholesterol synthesis. The requirement of the concerted action of multiple components of garlic led us to identify the active organosulfur compounds and their potency for inhibition of cholesterol synthesis (Liu and Yeh 2000). In this study, cultured rat hepatocytes were used to test the inhibition potency of organosulfur compounds derived from garlic. Included were water-soluble compounds, i.e., SAC, SEC, SPC, *S*-methylcysteine (SMC), γ-glutamyl *S*-allylcysteine (GSAC), γ-glutamyl *S*-methylcysteine (GSMC), γ-glutamyl *S*-propylcysteine (GSPC), S-allyl acetylcysteine (SAAC), *S*-allyl sulfonylalanine (SASA), *S*-allylmercaptocysteine (SAMC) and alliin. Lipid-soluble compounds were diallyl sulfide (DAS), DADS, diallyl trisulfide (DATS), dipropyl sulfide (DPS), dipropyl disulfide (DPDS) and methyl allylsulfide (MAS).

The cells were treated with [2-^{14}C]acetate in the presence or absence of the test compounds at 0.05–4.0 mmol/L for measurement of cholesterol synthesis. Among water-soluble compounds, S-alk(en)ylcysteines (i.e., SAC, SEC and SPC) exhibited dose-dependent inhibition on the rate of cholesterol synthesis with maximal 40–60% inhibition achieved at 2.0–4.0 mmol/L concentrations. Glutamate derivatives of S-alk(en)ylcysteines (i.e., GSAC, GSMC and GSPC) depressed the synthesis by 20–35%. Alliin, SAAC and SASA had no inhibitory effect. Lipid-soluble DAS, DADS, DATS, DPS and DPDS decreased the rate of cholesterol synthesis by 10–15% at concentrations of 0.05–0.5 mmol/L. At higher concentrations (i.e., 1.0, 2.0 and 4.0 mmol/L), DATS, DADS and DPDS, respectively, diminished the rate of [2-^{14}C] acetate incorporation into cholesterol. MAS did not affect the rate of cholesterol synthesis. Incubation of hepatocytes with each of the lipid-soluble sulfur compounds caused a dose-dependent increase in secretion of cellular lactate dehydrogenase (LDH) into the culture medium. More than 90% of total cellular LDH was released into the medium in the presence of high concentrations of DATS (1.0 mmol/L), DADS (2.0 mmol/L) and DPDS (4.0 mmol/L), suggesting that these compounds were cytotoxic. The cells incubated without the test compound released 13–16% of the cellular LDH. This level of LDH secretion was unaltered by the water-soluble sulfur compounds except for SAMC. Interestingly, SAMC at high concentrations (i.e., 2.0 and 4.0 mmol/L) also abolished the rate of cholesterol synthesis and was accompanied by ~80% secretion of the cellular LDH. These results suggest that the inhibition in cholesterol synthesis by water-soluble sulfur compounds is attributable to an impairment in the cholesterol synthetic pathway, whereas the inhibition by lipid-soluble compounds results from their potent cytotoxicity. However, it should be noted that the inhibition of cholesterol synthesis by a low concentration of DADS has been attributed to suppression of HMG-CoA reductase as well (Gebhardt and Beck 1996). Consistent with the present in vitro observation, different garlic preparations supplemented in animal diets were shown to depress hepatic synthesis of cholesterol (Qureshi et al. 1983a, 1983b and 1987).

The concentration-dependent inhibition of cholesterol synthesis permitted us to estimate the 50% inhibitory concentration (IC_{50}) for water-soluble compounds. The maximal inhibition of cholesterol synthesis by SAC, SPC, SEC, GSAC and GSPC was 62, 51, 45, 38, 21, and 21%, respectively, and the calculated IC_{50} was 1.22, 0.34, 1.12, 0.66, 1.75 and 1.88 mol/L, respectively. It should be pointed out that SAC is the major water-soluble sulfur compound of garlic. Although SAC content might vary from one preparation to another, AGE has been reported to contain 456 μg SAC/g powder (Amagase and Milner 1993). On the bases of the maximal inhibition, the IC_{50} and the concentration present in AGE, SAC could be the major sulfur compound responsible for the cholesterol-lowering effect seen in our animal feeding study and human intervention study.

Summary

We have demonstrated the cholesterol-lowering effects of AGE in both humans and rats. Although the mechanism(s) is not completely understood, the data from our animal study indicate that the hypocholesterolemic action of garlic stems in part from inhibition of hepatic cholesterol synthesis. Our in vitro studies further revealed that water-soluble organosulfur compounds, especially SAC, are potent inhibitors of cholesterol synthesis, and hence may be the major principles of garlic responsible for the reduction of plasma cholesterol level.

ACKNOWLEDGMENTS

The authors are thankful for technical expertise provided by Shelley Evans and Shaw-Mei Yeh.

LITERATURE CITED

Amagase, H. & Milner, J. A. (1993) Impact of various sources of garlic and their constituents on 7, 12-dimethylbenz[a]anthracene binding to mammary cell DNA. Carcinogenesis 14: 1627–1631.

American Institute of Nutrition (1977) Report of the American Institute of Nutrition ad hoc committee on standards for nutritional studies. J. Nutr. 107: 1340–1348.
Arora, R. C. & Arora, S. (1981) Comparative effect of clofibrate, garlic and onion on alimentary hyperlipemia. Atherosclerosis 39: 447–452.
Arora, R. C., Arora. S. & Gupta, R. K. (1981) The long-term use of garlic in ischemic heart disease-an appraisal. Atherosclerosis 40: 175–179.
Berthold, H. K., Sudhop. T. & von Bergmann, K. (1998) Effect of a garlic oil preparation on serum lipoproteins and cholesterol metabolism: a randomized controlled trail. J. Am. Med. Assoc. 279: 1900–1902.
Block, E. (1985) The chemistry of garlic and onions: a number of curious sulfur compounds underlie the odor of garlic and the crying brought on by slicing an onion. The compounds also account for medical properties long ascribed to garlic and onions. Sci. Am. 252: 114–119.
Bordia, A. (1981) Effect of garlic on blood lipids in patients with coronary heart disease. Am. J. Clin. Nutr. 34: 2100–2103.
Bordia, A., Arora, S. K., Kothari, L. K., Jain, K. C, Rathore, B. S., Rathore, A. S., Dube, M. K. & Bhu, N. (1975) The protective action of essential oils of onion and garlic in cholesterol-fed rabbits. Atherosclerosis 22: 103–109.
Bordia, A. & Verma, S. K. (1980) Effect of garlic feeding on regression of experimental atherosclerosis in rabbits. Artery 7: 428–437.
Buiatti, E., Palli, D., Blanchi, S., Decarli, A., Amadori, D., Avellini, C., Cipriani, F., Cocco, P., Giacosa, A., Lorenzini,L., Marubini, E., Puntoni, R., Saragoni, A., Fraumeni, J., Jr. & Blot, W. (1991) A case-control study of gastric cancer and diet in Italy. III, Risk patterns by histologic type. Int. J. Cancer. 48: 369–374.
Chang, M. W. & Johnson, M. A. (1980) Effect of garlic on carbohydrate metabolism and lipid synthesis in rats. J. Nutr. 110: 931–936.
Chi, M. S. (1982) Effects of garlic products on lipid metabolism in cholesterol-fed rats. Proc. Soc. Exp. Biol. Med. 171: 174–178.
Chi, M. S., Koh, E. T. & Steward, T. J. (1982) Effects of garlic on lipid metabolism in rats fed cholesterol or lard. J. Nutr. 112: 241–248.
Dausch, J. G. & Nixon, D. W. (1990) Garlic: a review of its relationship to malignant disease. Prev. Med. 19: 346–361.
Dietschy, J. M., Woollett, L. A. & Spady, D. K. (1993) The interaction of dietary cholesterol and specific fatty acids in the regulation of LDL receptor activity and plasma LDL-cholesterol concentrations. Ann. N.Y. Acad. Sci. 676: 11–26.
Ernst, E. (1987) Cardiovascular effects of garlic (*Allium sativum*): a review. Pharmatherapeutica 5: 83–89.
Gebhardt, R. (1993) Multiple inhibitory effects of garlic extracts on cholesterol biosynthesis in hepatocytes. Lipids 28: 613–619.
Gebhardt, R. & Beck, H. (1996) Differential inhibitory effects of garlic-derived organosulfur compounds on cholesterol biosynthesis in primary rat hepatocyte cultures. Lipids 31: 1269–1276.
Grundy, S. M. (1986) Cholesterol and coronary heart disease. A new era. J. Am. Med. Assoc. 256: 2849–2859.
Han, J. (1993) Highlights of the cancer chemoprevention studies in China. Prev. Med. 22: 712–722.
Isaacsohn, J. L., Moser, M., Stein, E. A., Dudley, K., Davey, J. A., Liskov, E. & Black, H. R. (1998) Garlic powder and plasma lipids and lipoproteins. Arch. Intern. Med. 158: 1189–1194.
Itokawa, Y., Inoue, K., Sasagawa, S. & Fujiwara, M. (1973) Effect of *S*-methylcysteine sulfoxide, *S*-allycysteine sulfoxide and related amino acids on lipid metabolism of experimental hypercholesterolemic rats. J. Nutr. 103: 88–92.
Jain, A. K., Vargas, R., Gotzkowsky, S. & McMahon, F. G. (1993) Can garlic reduce levels of serum lipids? A controlled clinical study. Am. J. Med. 94: 632–635.
Kamanna, V. S. & Chandrasekhara, N. (1982) Effects of garlic (*Allium sativum* linn) on serum lipoproteins and lipoprotein cholesterol levels in albino rats rendered hypercholesterolemic by feeding cholesterol. Lipids 17: 483–488.
Kiesewetter, H., Jung, F., Jung, E. M., Mroweitz, C., Koscielny, J. & Wenzel, E. (1993) Effect of garlic on platelet aggregation in patients with increased risk of juvenile ischaemic attack. Eur. J. Clin. Pharm. 45: 333–336.
Lau, B.H.S., Lam, F. & Wang-Chen, R. (1987) Effect of an odor-modified garlic preparation on blood lipids. Nutr. Res. 7: 139–149.
Lin, X. Y., Liu, J. Z. & Milner, J. A. (1994) Dietary garlic suppresses DNA adducts caused by N-nitroso compounds. Carcinogenesis 15: 349–352.
Liu, J., Lin, R. I. & Milner J. A. (1992) Inhibition of 7,12-dimethylbenz[a]anthracene-induced mammary tumors and DNA adducts by garlic powder. Carcinogenesis 13: 1847–1851.
Liu, L. & Yeh, Y. Y. (2000) Inhibition of cholesterol biosynthesis by organosulfur compounds derived from garlic. Lipids 35: 197–203.
Mathew, B. C., Daniel, R. S. & Augusti, K. T. (1996) Hypolipidemic effect of garlic protein substituted for casein in diet of rats compared to those of garlic oil. Indian J. Exp. Biol. 34: 337–340.
Mei, X., Lin, X., Liu, J., Lin, X, Song. P., Hu, J. & Liang, X. (1989) The blocking of garlic on the formation of *N*-nitrosoproline in humans. Acta. Nutr. Sin. 11: 141–145.
Qureshi, A. A., Abuirmeileh, N., Din, Z. Z., Elson, C. E. & Burger, W. C. (1983a) Inhibition of cholesterol and fatty acid biosynthesis in liver enzymes and chicken hepatocytes by polar fractions of garlic. Lipids 18: 343–348.
Qureshi, A. A., Din, Z. Z., Abuirmeileh, N., Burger, W. C., Ahmad, Y. & Elson, C. E. (1983b) Suppression of avian hepatic lipid metabolism by solvent extracts of garlic: impact on serum lipids. J. Nutr. 113: 1746–1755.
Qureshi, A. A., Crenshaw, T. D., Abuirmeileh, N., Peterson, D. M. & Elson, C. E. (1987) Influence of minor plant constituents on porcine hepatic lipid metabolism: impact on serum lipid. Atherosclerosis 64: 109–115.
Sakamoto, K., Lawson, L. D. & Milner, J. A. (1997) Allyl sulfides from garlic suppress the in vitro proliferation of human A549 lung tumor cells. Nutr. Cancer 29: 152–156.
Schaffer, E. M., Liu, J. Z., Green, J., Dangler, C. A. & Milner, J. A. (1996) Garlic and associated allyl sulfur components inhibit *N*-methyl-*N*-nitrosourea induced rat mammary carcinogenesis. Cancer Lett. 102: 199–204.
Sendl, A., Schliack, M., Loser, R., Stanislaus, F. & Wagner, H. (1992) Inhibition of cholesterol synthesis in vitro by extracts and isolated compounds prepared from garlic and wild garlic. Atherosclerosis 94: 79–85.
Silagy, C. & Neil, A. (1994a) A meta-analysis of the effect of garlic on blood pressure. J. Hypertens. 12: 463–468.
Silagy, C. & Neil, A. (1994b) Garlic as a lipid lowering agent—a meta-analysis. J. R. Coll. Physicians Lond. 28: 39–45.
Simons, L. A., Balasubramanian, S., von Konigsmark, M., Parfitt, A., Simons, J. & Peters, W. (1995) On the effect of garlic on plasma lipids and lipoproteins in mild hypercholesterolemia. Atherosclerosis 113: 219–225.
Spady, D. K. & Dietschy, J. M. (1988) Interaction of dietary cholesterol and triglycerides in the regulation of hepatic low density lipoprotein transport in the hamster. J. Clin. Investig. 81: 300–309.
Srivastava, K. C. & Tyagi, O. D. (1993) Effects of a garlic-derived principle (ajoene) on aggregation and arachidonic acid metabolism in human blood platelets. Prostaglandins Leukot. Essent. Fatty Acids 49: 587–595.
Steiner, M., Khan, A. H., Holbert, D. & Lin, R. I. (1996) A double-blind crossover study in moderately hypercholesterolemic men that compared the effect of aged garlic extract and placebo administration on blood lipids. Am. J. Clin. Nutr. 64: 866–870.
Steinmetz, K. A., Kushi, L. H., Bostick, R. M., Folson, A. R. & Potter, I. D. (1994) Vegetables, fruit and colon cancer in the Iowa Women's Health Study. Am. J. Epidemiol. 39: 1–15.
Sumiyoshi, H. & Wargovich, M. (1990) Chemoprevention of 1,2-dimethylhydrazine-induced colon cancer in mice by naturally occurring organosulfur compounds. Cancer Res. 50: 5084–5087.
Sundaram, S. G. & Milner, J. A. (1996) Diallyl disulfide inhibits the proliferation of human tumor cells in culture. Biochim. Biophys. Acta 1315: 15–20.
Tadi, P. P., Teel, R. W. & Lau, B.H.S. (1990) Anticandidal and anticarcinogenic potentials of garlic. Int. Clin. Nutr. Rev. 10: 423–429.
Warshafsky, S., Kamer, R. S. & Sivak, S. L. (1993) Effect of garlic on total serum cholesterol. A meta-analysis. Ann. Intern. Med. 119: 599–605.
Weinberg, D. S., Manier, M. L., Richardson, M. D. & Haibach, F. G. (1993) Identification and quantification of organosulfur compliance markers in a garlic extract. J. Agric. Food Chem. 41: 37–41.
Yamasaki, T., Li, L. & Lau, B. (1994) Garlic compounds protect vascular endothelial cells from hydrogen peroxide-induced oxidant injury. Phytother. Res. 8: 408–412.
Yeh, Y. Y. & Yeh, S. M. (1994) Garlic reduces plasma lipids by inhibiting hepatic cholesterol and triacylglycerol synthesis. Lipids 29: 189–193.
Yeh, Y. Y., Lin, R. I., Yeh, S. M. & Evens, S. (1997) Garlic reduces plasma cholesterol in hypercholesterolemic men maintaining habitual diets. In: Food Factors for Cancer Prevention (Ohigashi, H., Osawa, T., Terao, J., Watanabe, S. & Toshikawa, T., eds.), pp. 226–230. Springer, Tokyo, Japan.
Zimmerman, W. & Zimmerman, B. (1990) Reduction in elevated blood lipids in hospitalized patients by a standardized garlic preparation. Br. J. Clin. Pract. 44: 20–23.
Zheng, W., Blot, W. J., Shu, X. O., Gao, Y. T., Ji, B. T., Ziegler, R. G. & Fraumeni, F., Jr. (1992) Diet and other risk factors for laryngeal cancer in Shanghai, China. Am. J. Epidemiol. 130: 178–191.